45th International Conference on Environmental Systems

Miniature Liquid Chromatographic Systems for Human and Robotic Missions

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Pasadena, CA

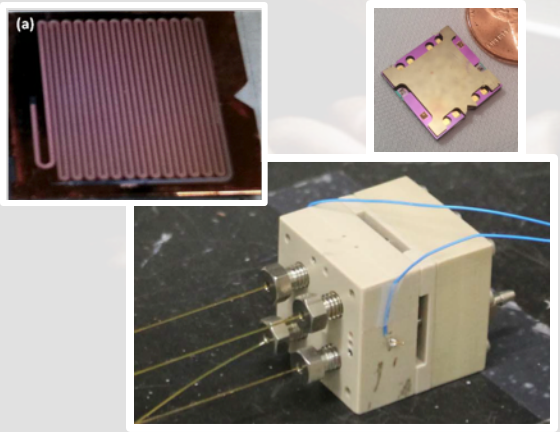
- SMD (Science Mission Directorate): Our instruments are meant to address two fundamental questions in *Astrobiology*, namely, "How does life begin and evolve?" and "Does life exist elsewhere in the Universe?" For these reasons, the *Science Objectives* for the analyzer are to look for:
  - ✓ Signs of extinct life by detecting Carboxylic Acids and Lipids - the longevity and preservation of carboxylic acids and lipids offer a chemical insight into potential primordial biological activity.
  - ✓ Extant life by searching for Peptides and Proteins - macromolecules that strongly indicate a biotic origin.
- Provide organic molecular detection and life detection capabilities for future *landed* missions to Mars, Europa, Titan, Enceladus, and other planetary bodies.
- Astrobiology field research on Earth.
- HEOMD (Human Exploration & Operations Mission Directorate): Experiments on ISS, astronaut health monitoring, environmental monitoring.
- Of the three main chromatographic technologies (GC, CE, LC), liquid chromatography is the least advanced.

JPL/Caltech MEMS-based Chromatography

Gas Chromatography (GC)

- Can be coupled to a MS
- Very fast
- Can separate volatile small molecules
- Need to derivatize amino acids, fatty acids, etc.

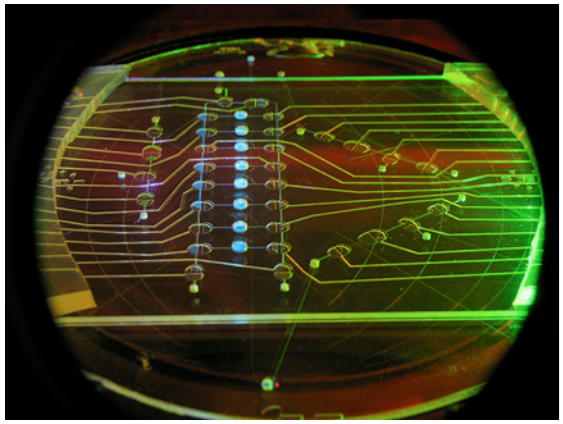
Madzunkov, S.M., MacAskill, J.A., Simcic, J., Kidd, R.D. & Darrach, M. (2013). Recent developments in gas chromatographs and mass spectrometers for crewed and robotic space missions. *J. Am. Inst. Aeronautics & Astronautics*. Electronic 2012-3453.



Capillary Electrophoresis (CE)

- Coupled to laser-based fluorescence detector
- Very sensitive/specific
- Can separate small molecules to macromolecules

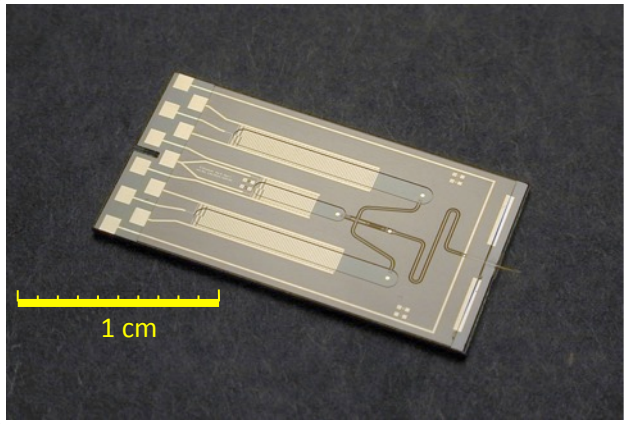
Willis, P. A., Stockton, A. M., Microchip Capillary Electrophoresis for In Situ Planetary Exploration. In *Capillary Electrophoresis and Microchip Capillary Electrophoresis*, John Wiley & Sons, Inc.: **2013**; pp 277-291.



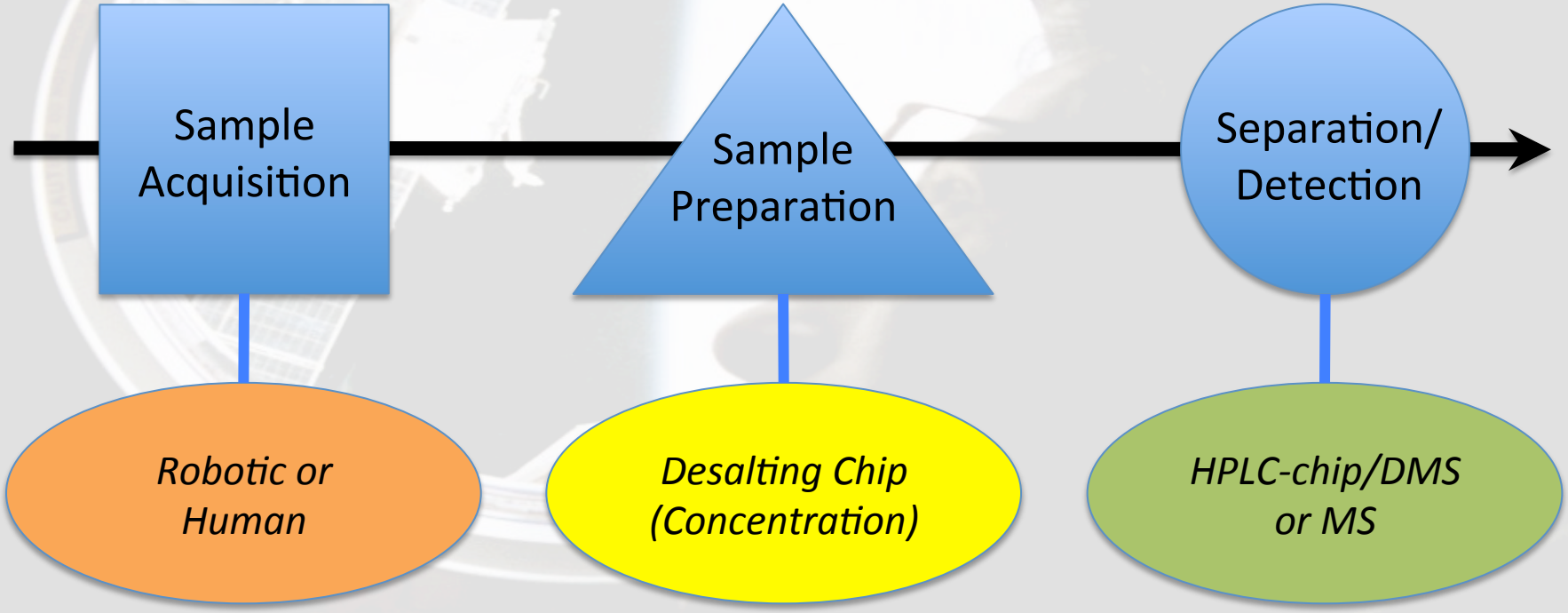
High Performance Liquid Chromatography (HPLC)

- Can be coupled to a MS
- Well suited for non-volatiles
- Can separate small molecules to macromolecules
- No derivatization required

Xie, J., Miao, Y., Shih, J., Tai, Y.-C. & Lee, T.D. (2005). Microfluidic platform for liquid chromatography-tandem mass spectrometry analyses of complex peptide mixtures. *Anal. Chem.* **77**, 6947-6953.



In designing *in situ*, liquid-based analytical instruments, three areas need to be addressed: *Sample Acquisition*, *Sample Preparation* and *Separation/Detection*.



We are pursuing research into all three areas focusing, thus far, on developing a unique, miniaturized solute analyzer based on microfluidics technology. This analyzer consists of an integrated microfluidics High Performance Liquid Chromatographic-chips coupled to either a Differential Mobility Spectrometer (µHPLC-chip/DMS) or Paul Ion Trap Mass Spectrometer (µHPLC-chip/MS)

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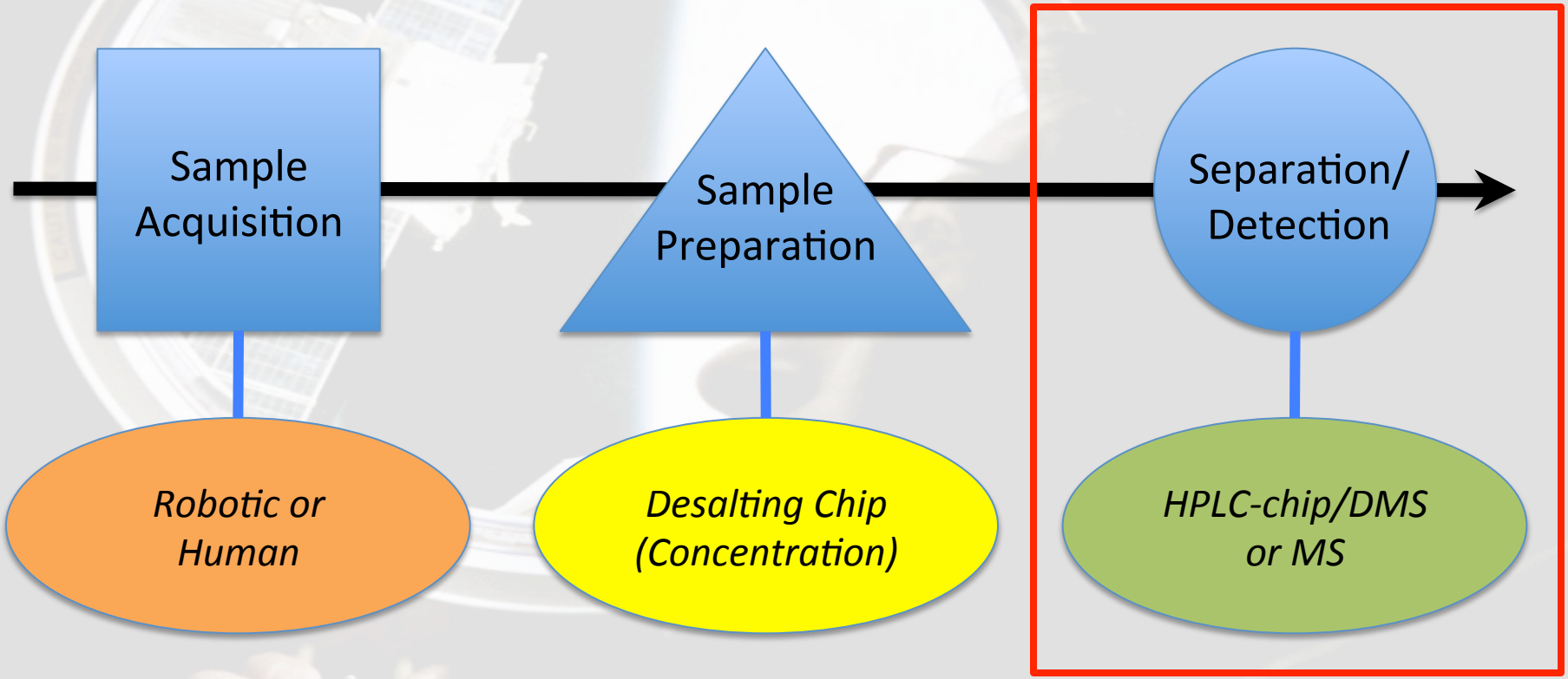

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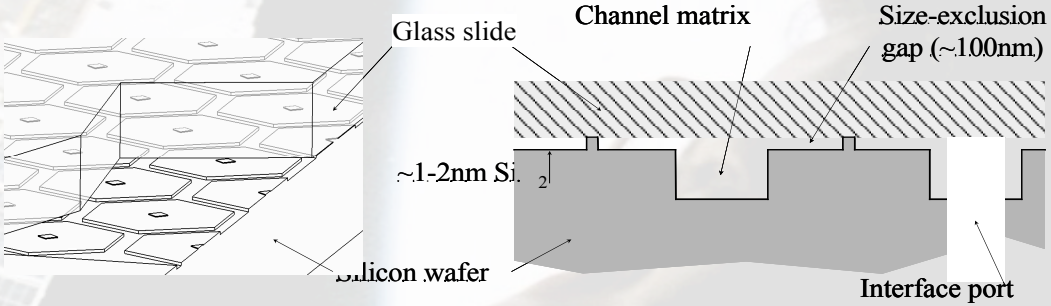
nanoSEC-chip/Laser-Induced Fluorescence Detector

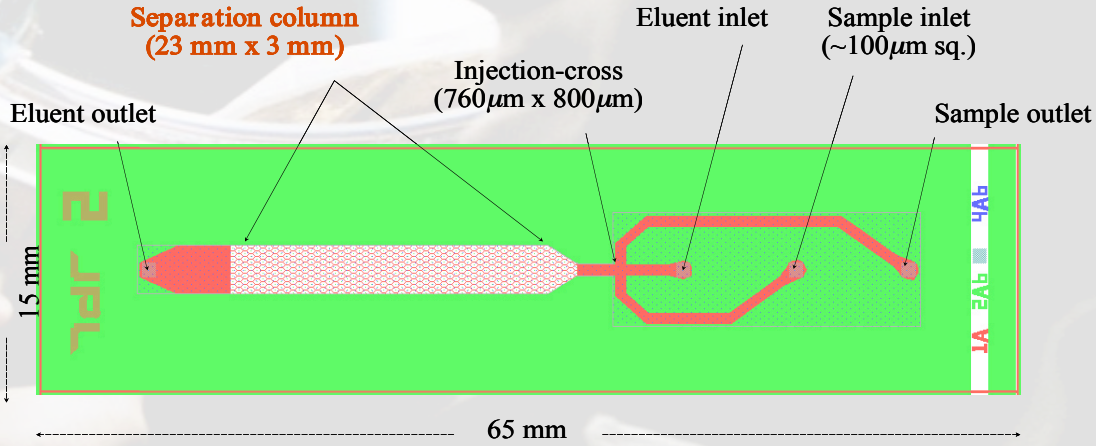
Collaborators: JPL's Micro Devices Laboratory

Target: fatty acids, small peptides, carbohydrates, kerogenic and humic fractions

Funding: NASA ROSES ASTID



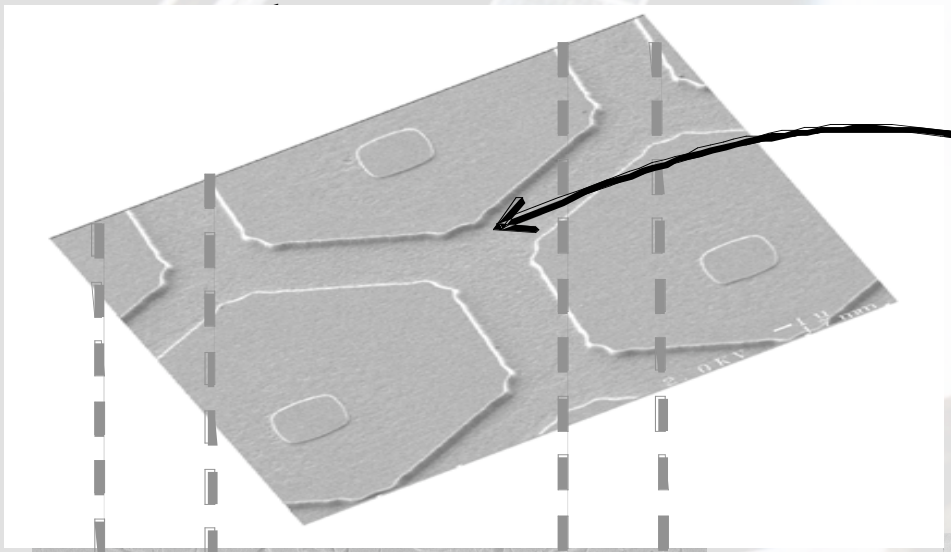
(A)



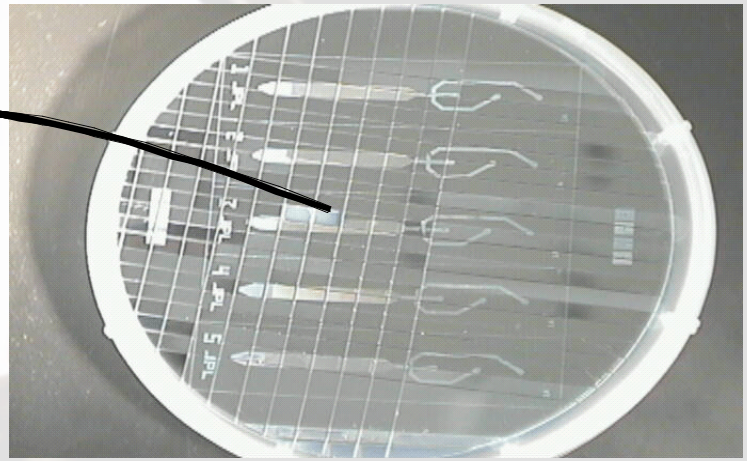
(B)

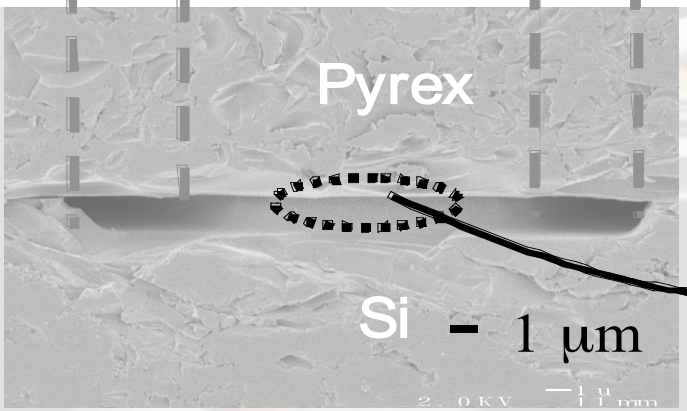
Figure 1: (A) 3-D and cross-sectional views of nSEC column schematics, (B) Top view of nSEC device layout

Top view of nSEC channels

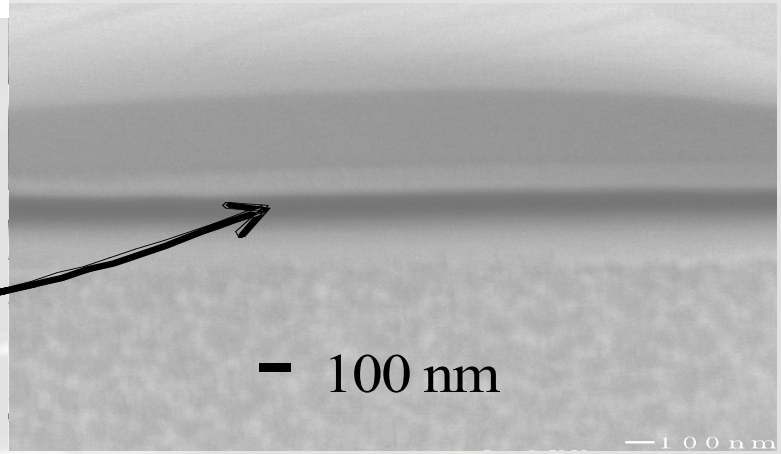


Sealed Si wafer containing five nSEC





Cross-section of bonded wafer showing 1 μm and 100 nm gaps



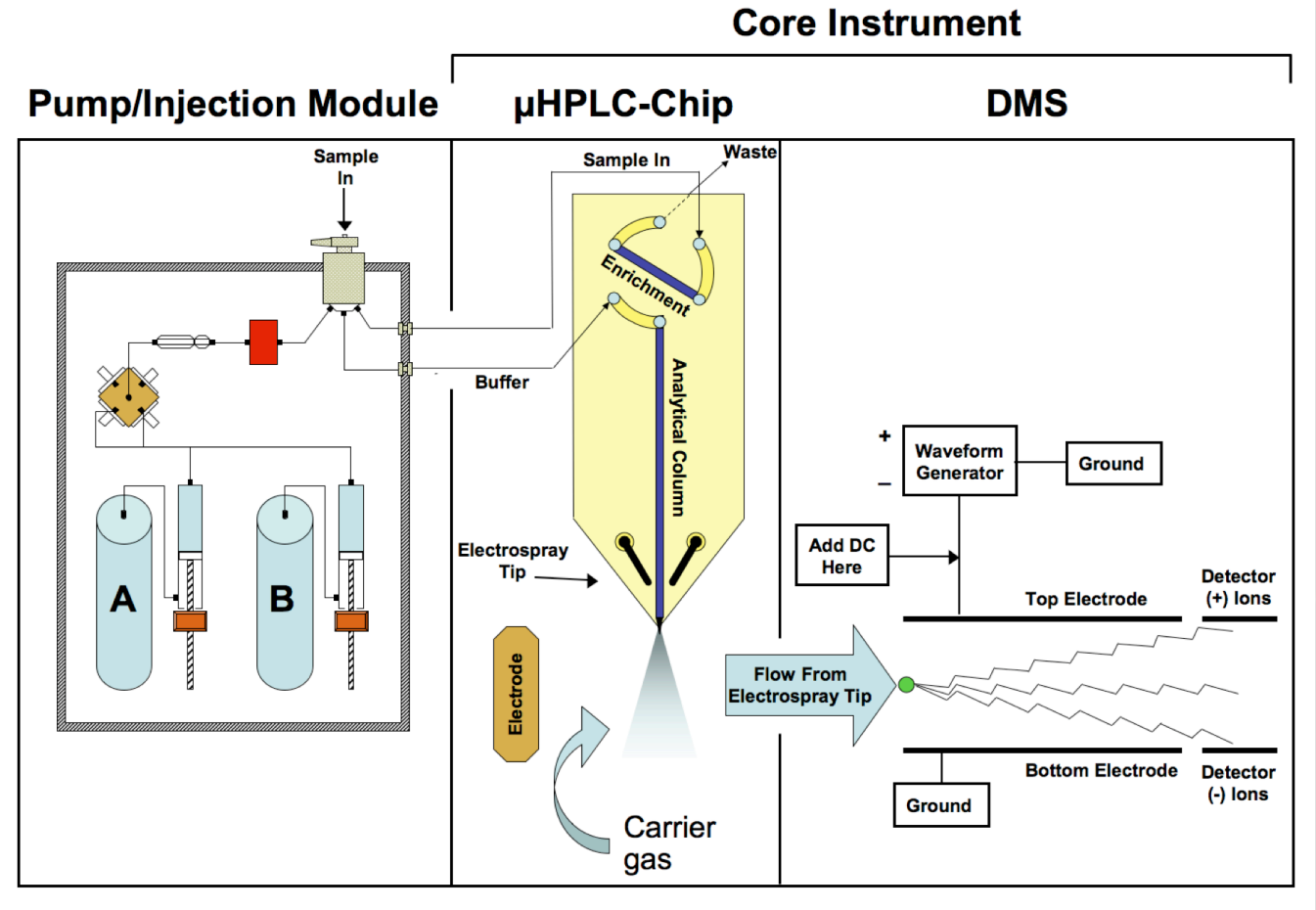
SEM image confirming presence of 100 nm gap in sealed nSEC device

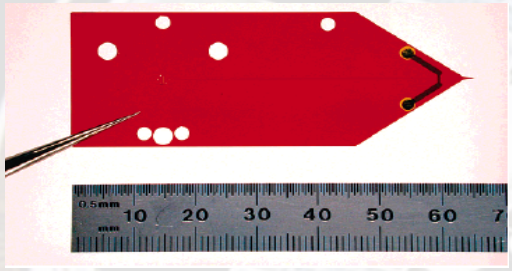
HPLC-chip/Differential Mobility Spectrometer (DMS)

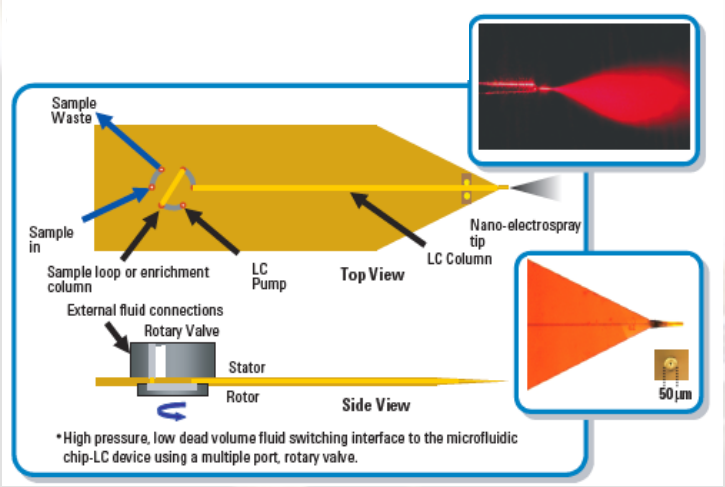
Collaborators: Agilent's Molecular Separations Lab & Sionex LLC

Target: fatty acids, small peptides, carbohydrates

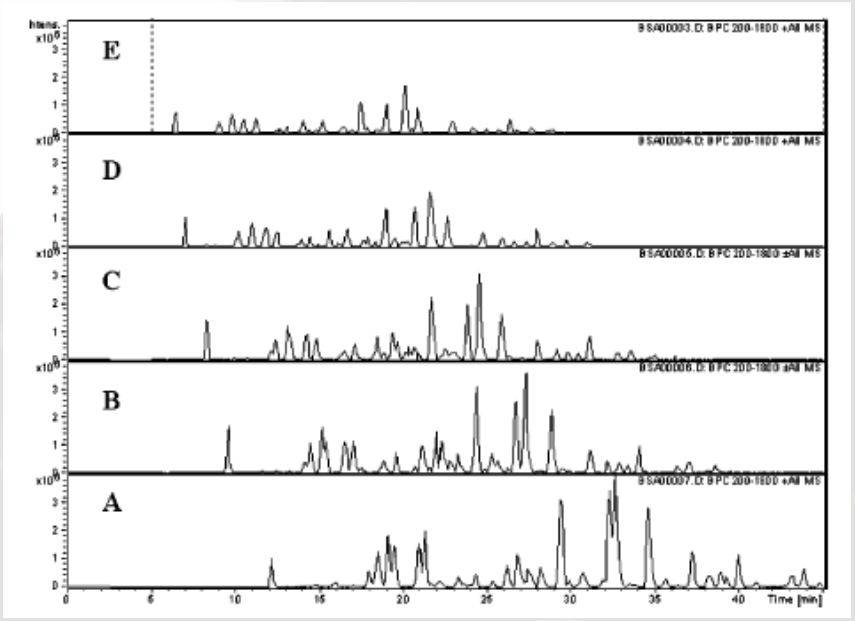
Funding: NASA ROSES PIDDP



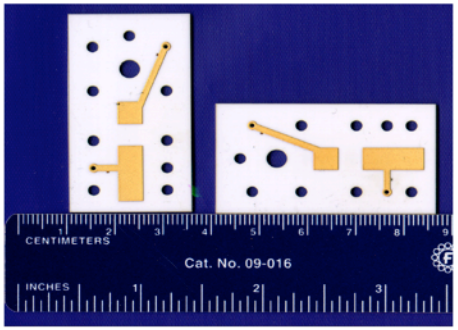




(Top) Photograph of the µHPLC-chip. The holes were used for alignment purposes. The dark pattern on the right end of the chip is the electrodeposited metal for contact to the fluid flow channel near the electrospray tip. (Bottom) Schematic top and side views of the µHPLC-chip



Base peak chromatograms of a 20 fmol tryptic digest of bovine serum albumin (BSA) running under different LC flow rate: (A) 100, (B) 150, (C) 200, (D) 300, and (E) 400 nL/min. The vertical scales in each spectrum are identical.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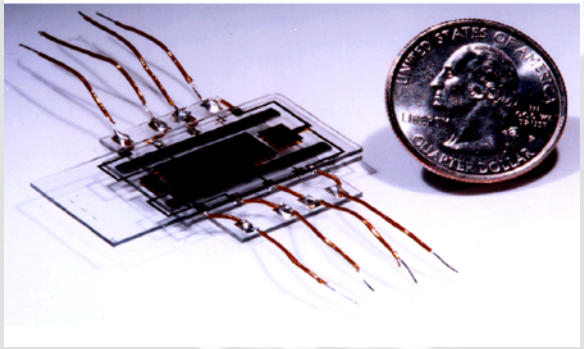
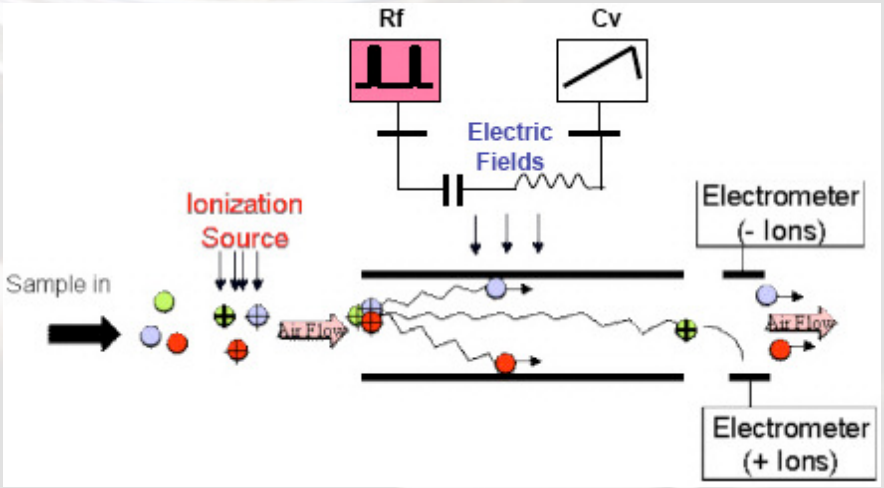



SIONEX
Corporation


Rf
Cv
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Electrometer
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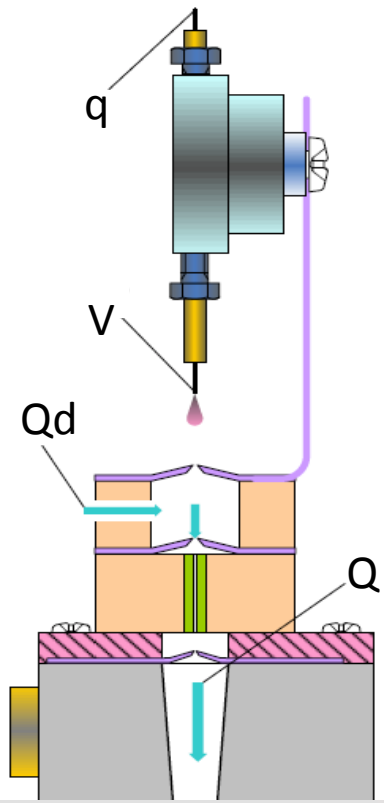
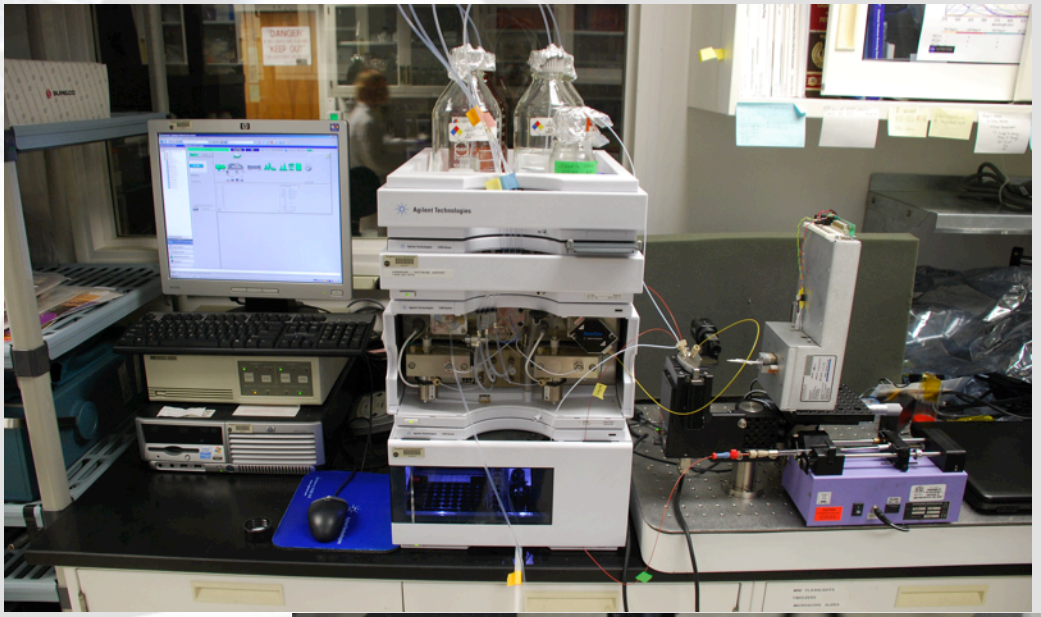
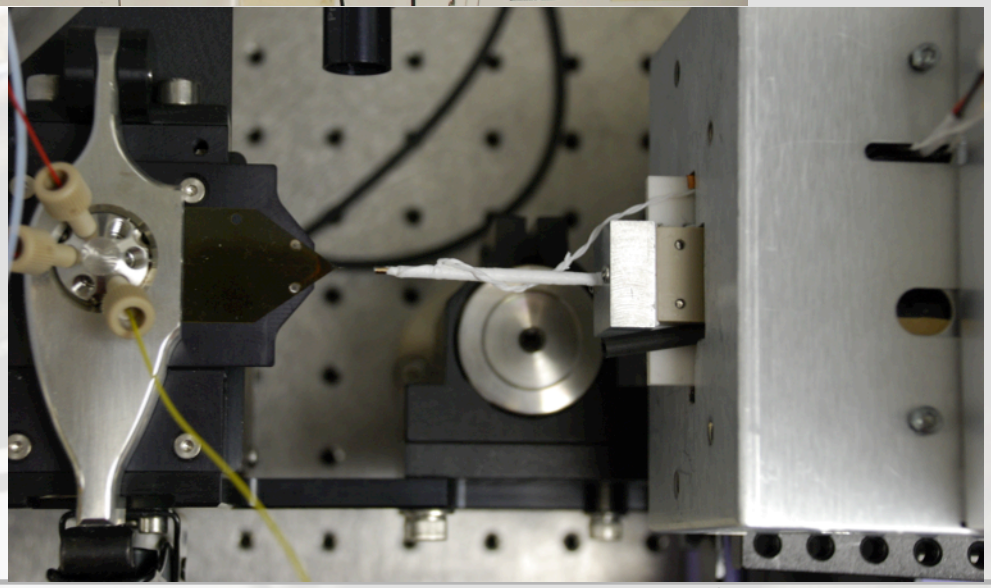
Preliminary HPLC electrospray to DMS

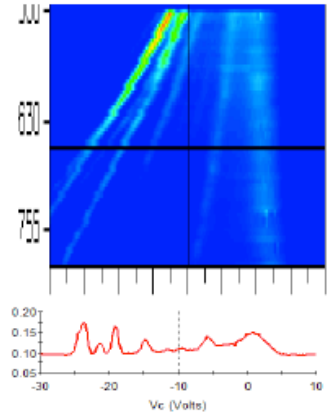
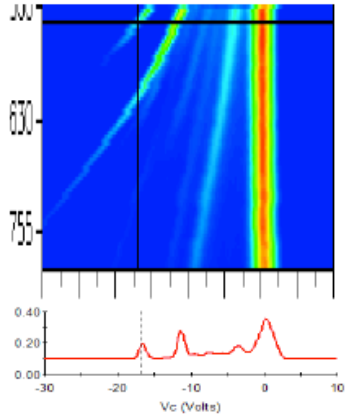
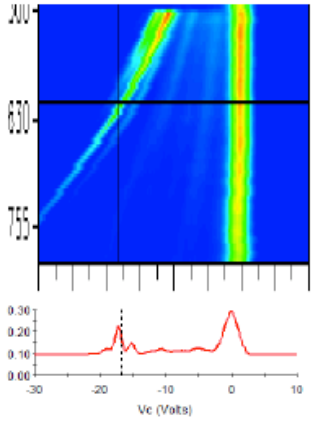
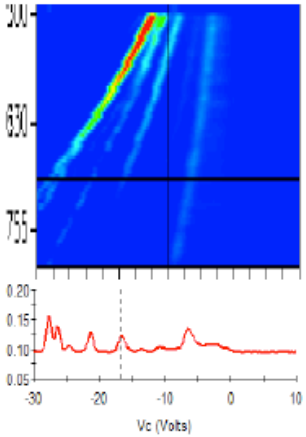
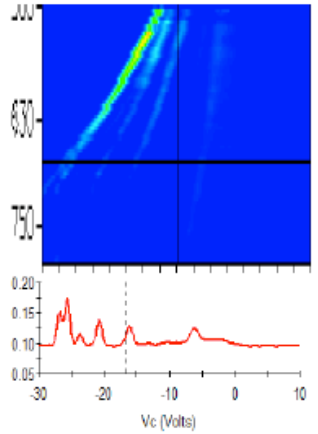
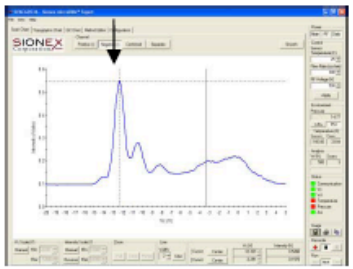
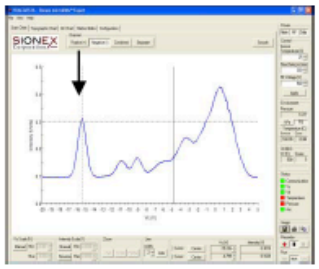
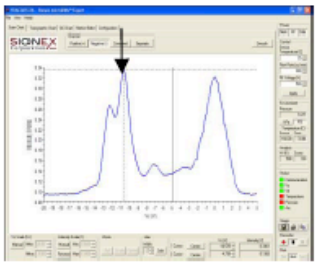
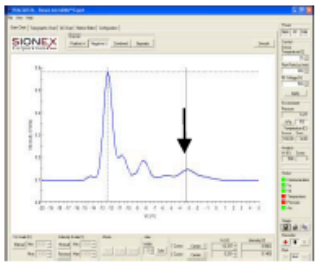
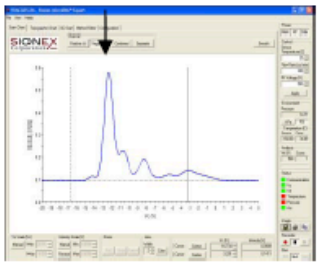






Coy, S.L. Krylov, E.V., Nazarov, E.G., Fornace Jr., A.J. & Kidd, R.D. (2013). Differential mobility spectrometry with nanospray ion source as a compact detector for small organics and inorganics. *Int. J. Ion Mobil. Spec.* 16, 217-227.

Target ions: fluoride – 37/19; chloride – 35; formate – 45; acetate – 59; bromated – 127/129. Mixture – 50+10+10+10+10 ppm. Q = 1 L/min; Qd = 10 L/hr; V = 1.9 kV; q = 800 nL/min; C_V = -25…5 V.

Solvent- Acetanitrale+water Solvent – acetonitrile+water	chloride – Cl^-	fluoride – $(H_2O)F^-$	bromate – BrO_3^-	Acetate- $C_2H_3O_2^-$
				
				
Vc=-12.3V	Vc==-15.5V	Vc=-10.6V	Vc=-3V	Vc=-12.3V

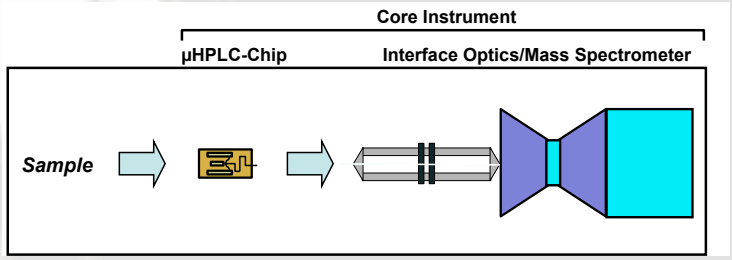
HPLC-chip/Mass Spectrometer (MS)

Co-Investigator: Y.-C. Tai, Caltech

Target: Carboxylic Acids & Lipids (fatty acids and sterols), Amino Acids & Peptides

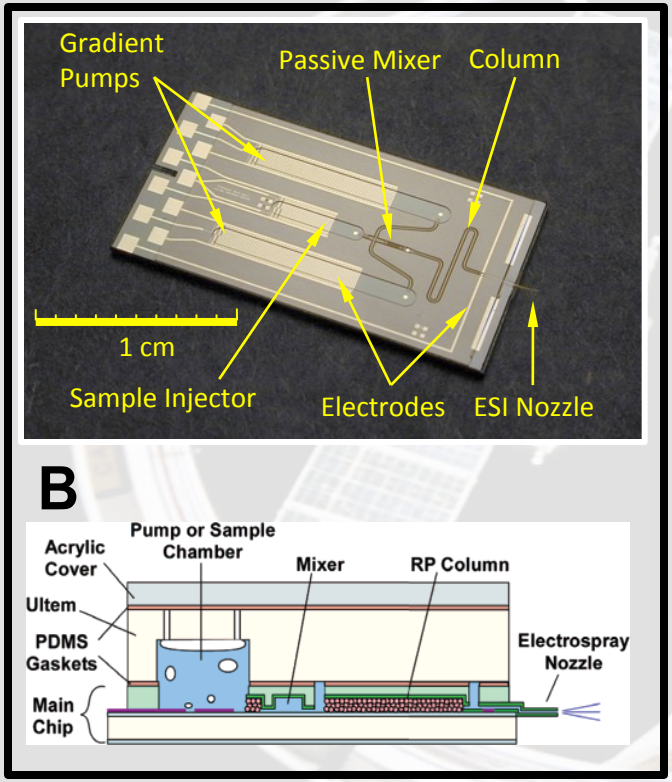
Funding: NASA ROSES ASTID

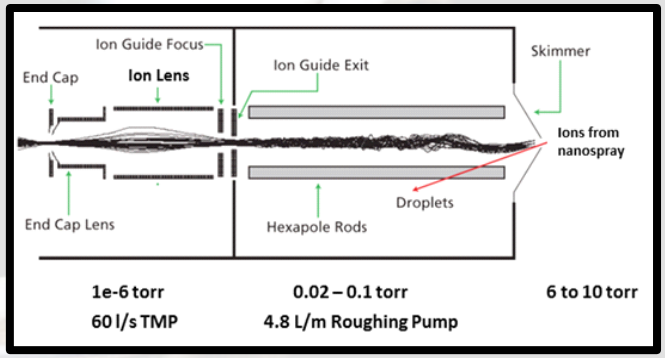
The ASTID NRA specifically solicits "**lab-in-a-teacup**" development projects. The goal is to apply micro/ nanotechnology to planetary instrumentation and develop highly integrated miniature instruments suites with the capability to address astrobiology questions in planetary exploration.

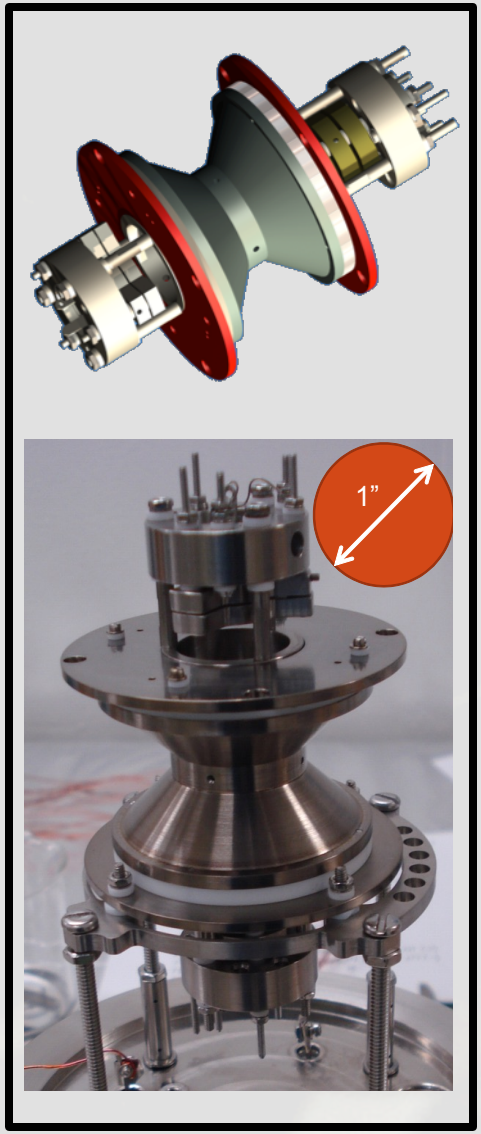


Capability Goals:

- ***Sensitivity:*** Low parts-per-billion/trillion (ppb/ppt) depending on species.
- ***Salt Compatibility:*** Desalting carried out by a second, ion-exchange HPLC-chip.
- ***Resolution:*** Capable of separating isomers and determining fatty acid chain lengths.
- ***Mass Range:*** From small organic acids (e.g. acetate) to macromolecules.
- ***Detect Unknowns:*** Yes, with no *a priori* selection of chemical species.
- ***Mass / Power:*** <6 kg / <20 watts for complete instrument based on current parameters for miniaturized HPLC-chip and MS instruments (under our funded PIDDP award).
- ***Chemical Derivatization / Labeling:*** None required.
- ***Expandability:*** Several chromatographic chips could be integrated together (see **Figure 1-7**) and serviced by single detection system.







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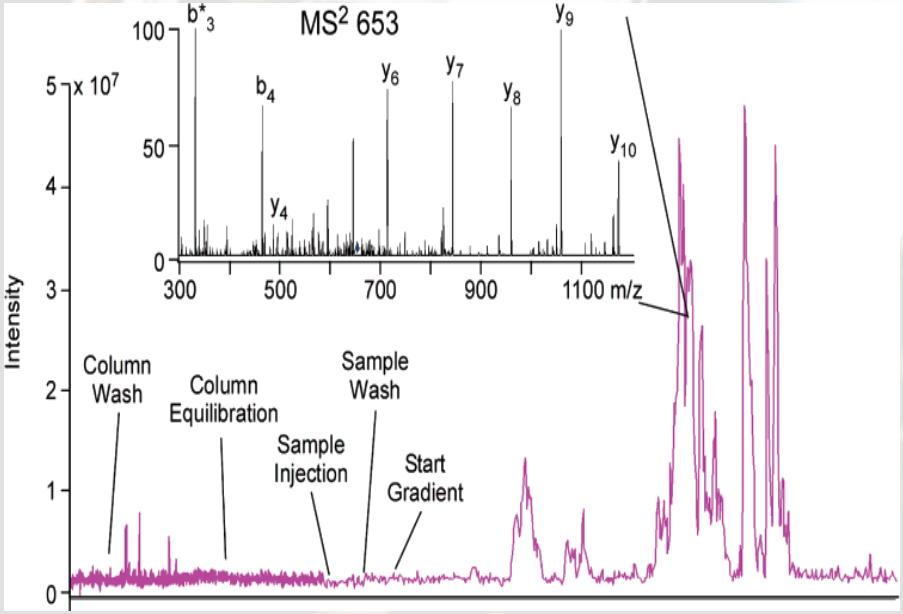
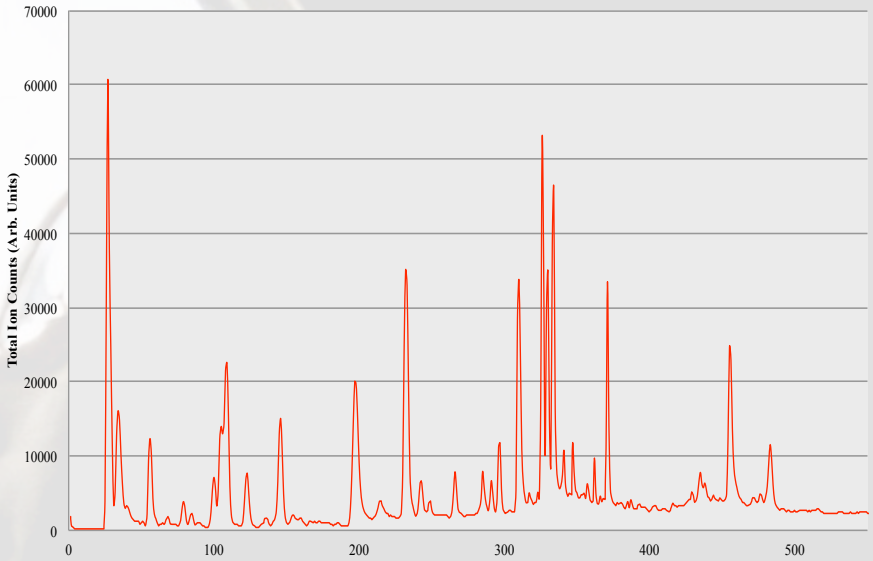
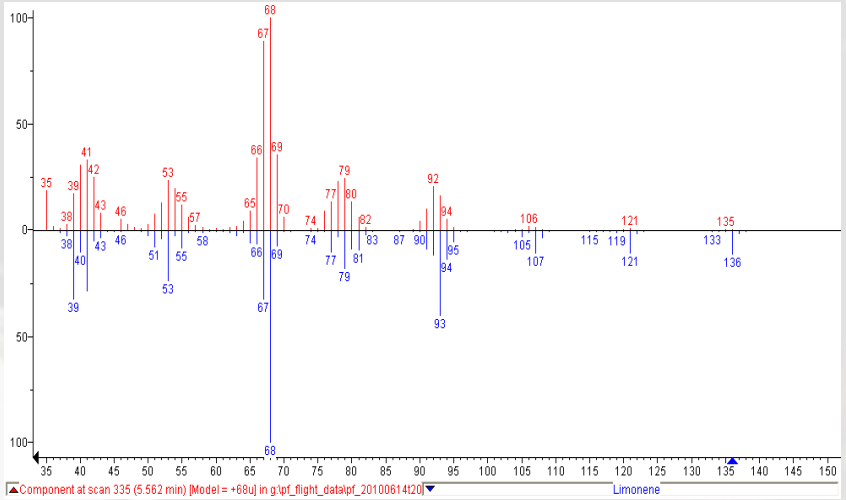
HPLC-chip/Agilent ion trap MS

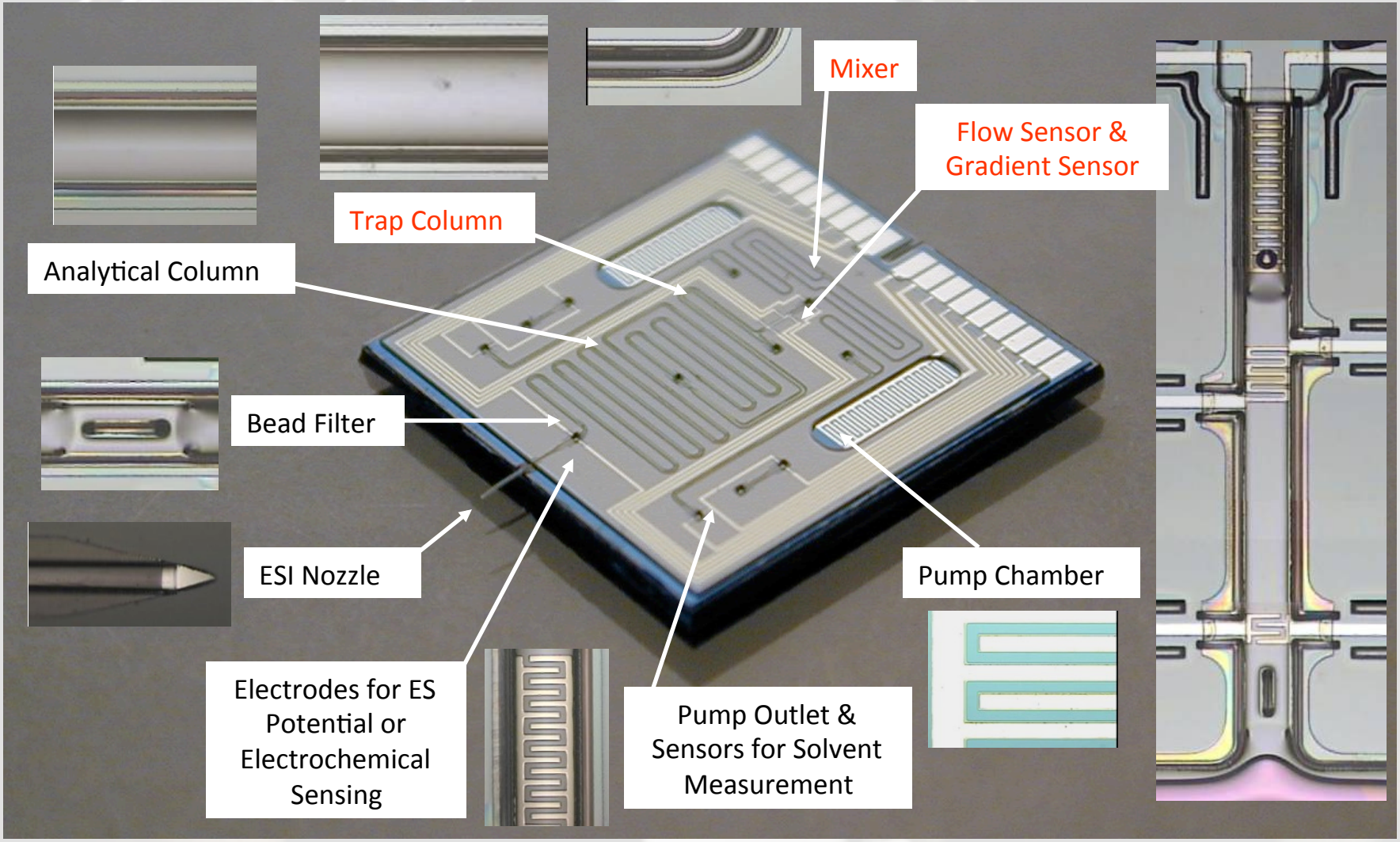


JPL ion trap MS

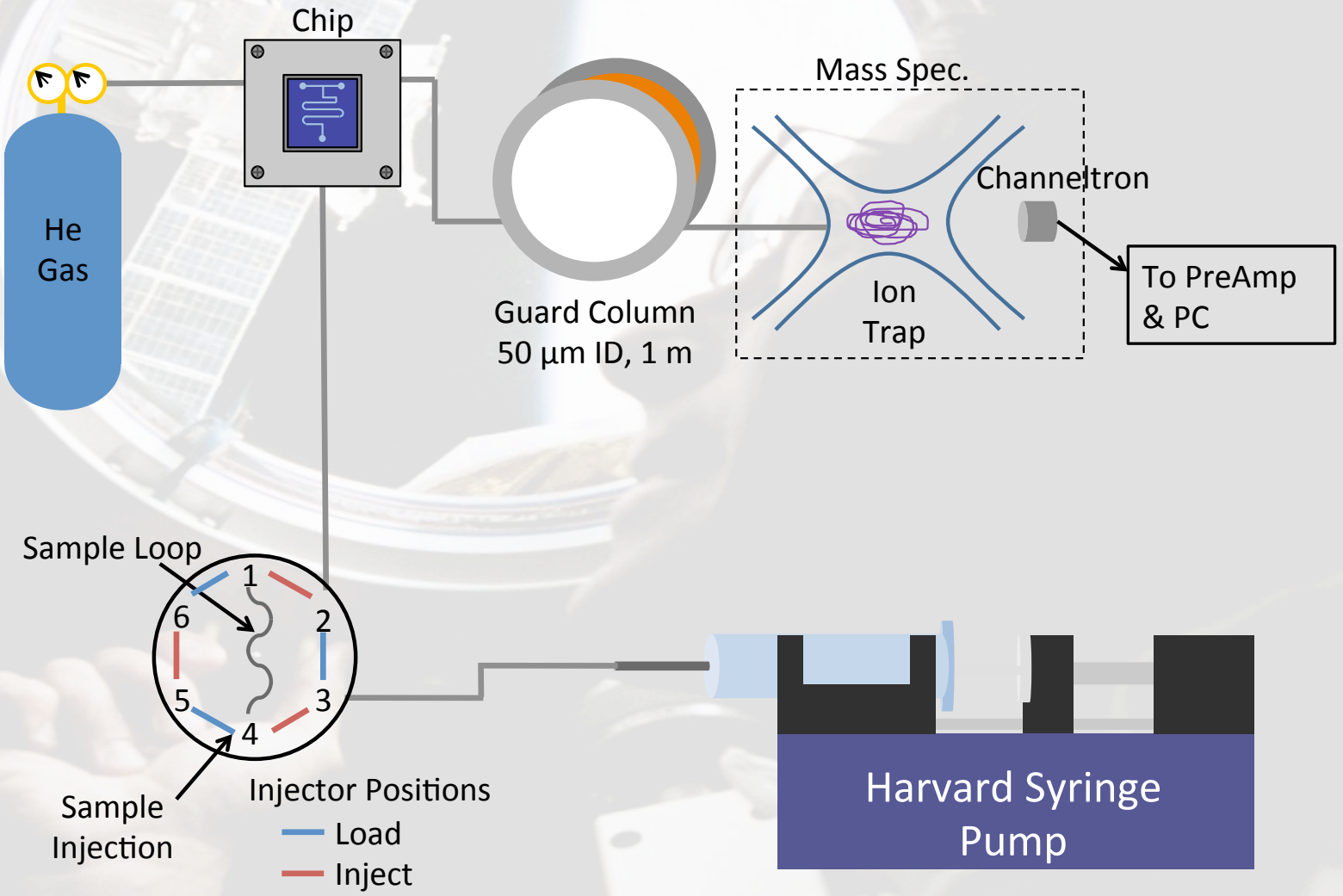


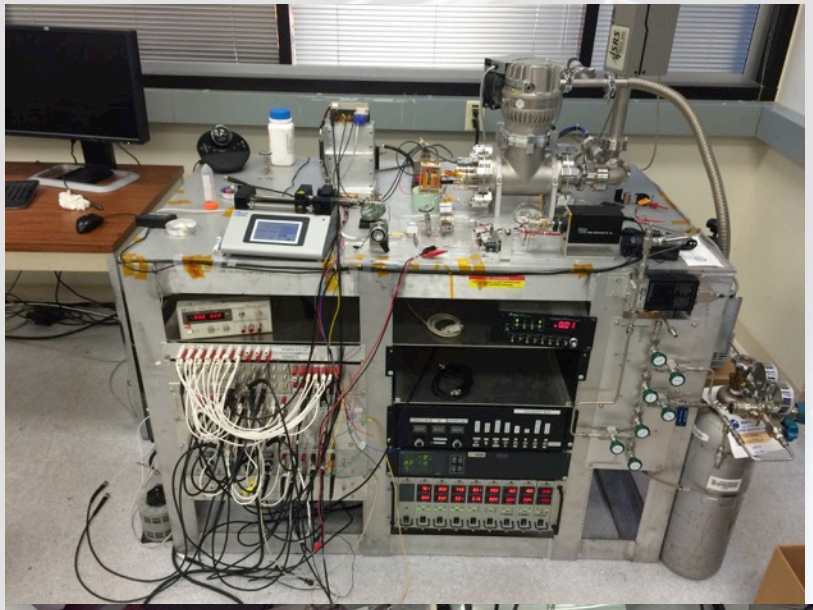


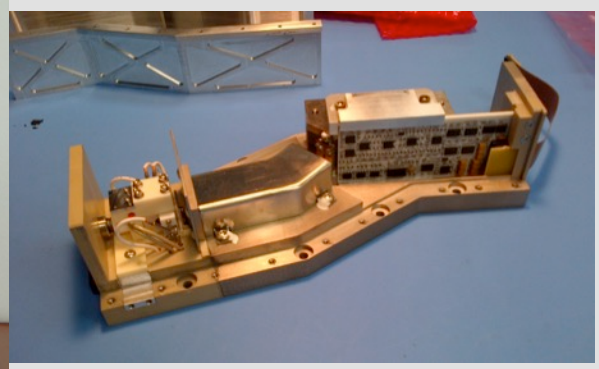
uHPLC

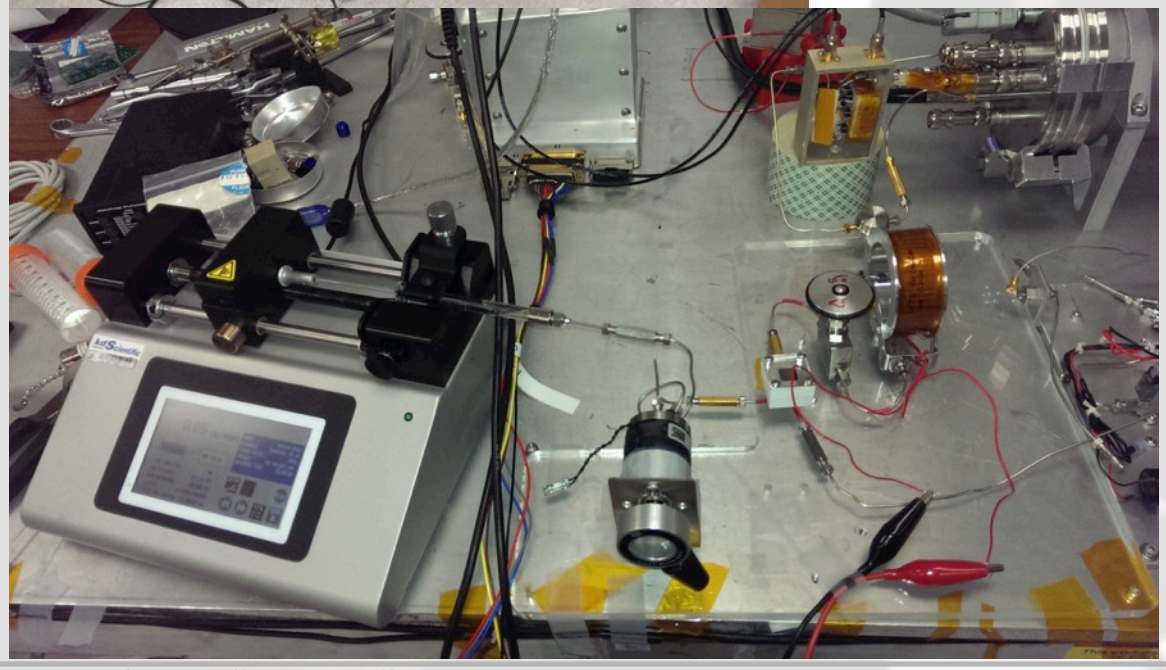

Mixer
Flow Sensor & Gradient Sensor
Trap Column
Analytical Column
Bead Filter
ESI Nozzle
Pump Chamber
Electrodes for ES Potential or Electrochemical Sensing
Pump Outlet & Sensors for Solvent Measurement

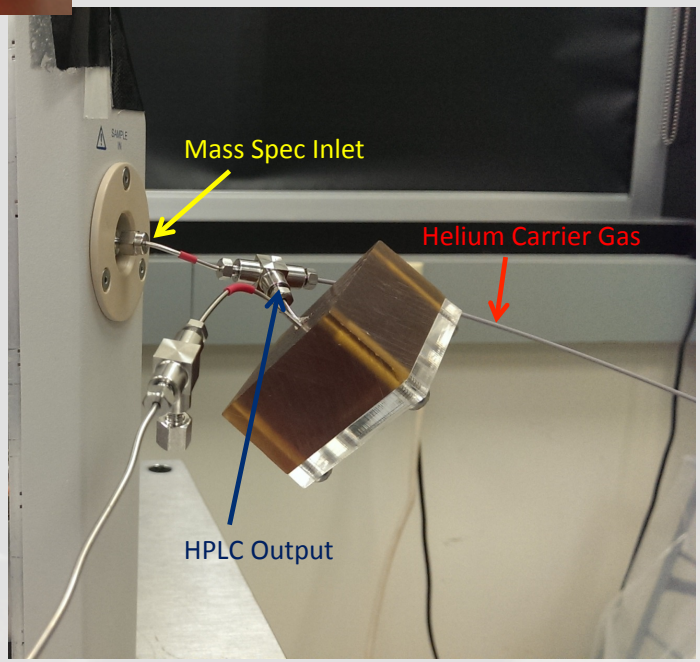
Since the flow rate of the HPLC-chip is so low, ~20-50 nL/min, we explored direct entry (no ESI) into Paul ion trap and magnetic sector mass spectrometers.

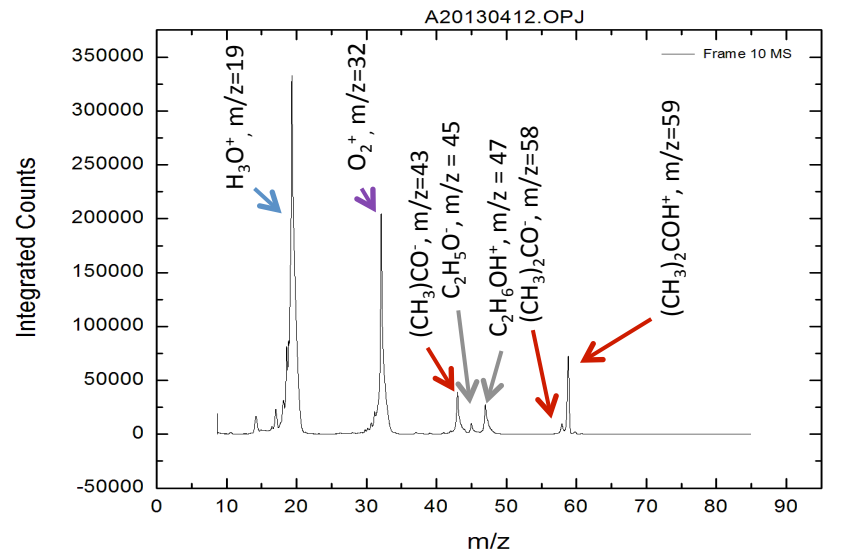


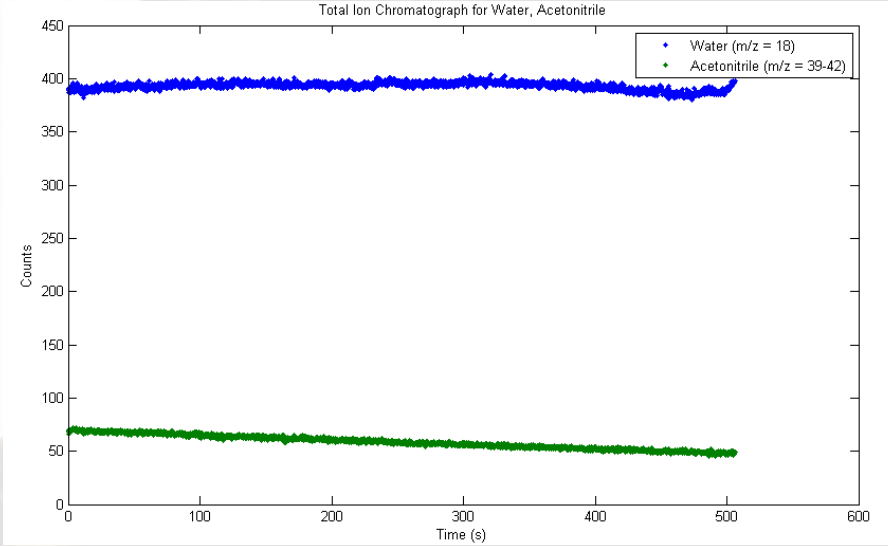


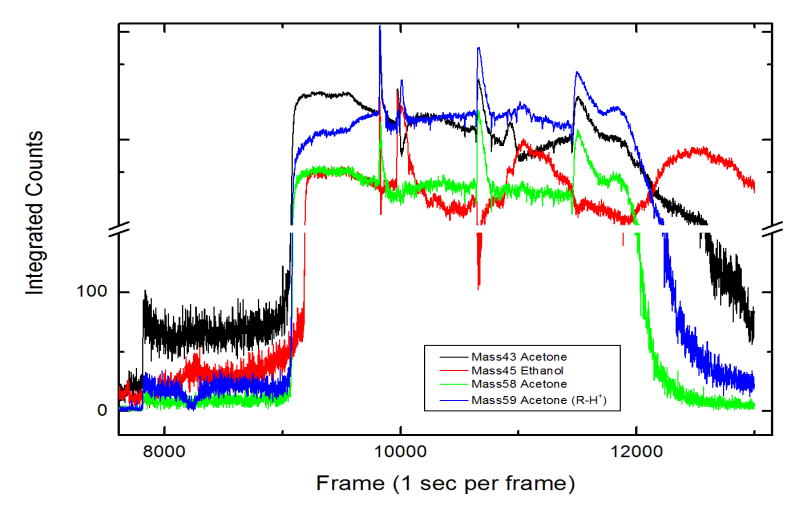


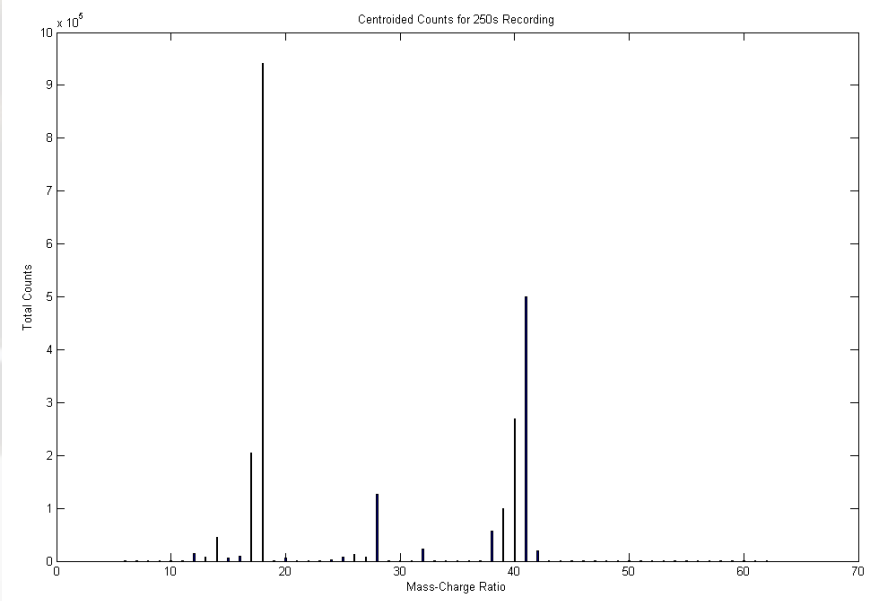


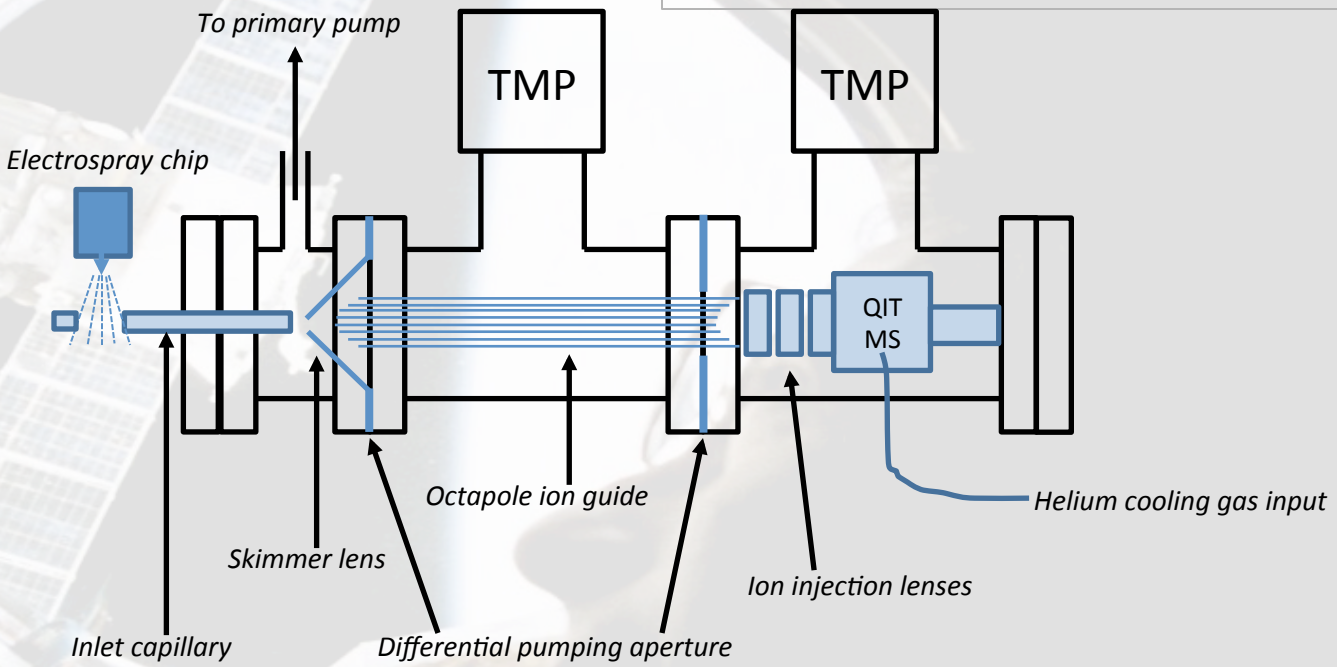

Mass Spec Inlet
Helium Carrier Gas
HPLC Output

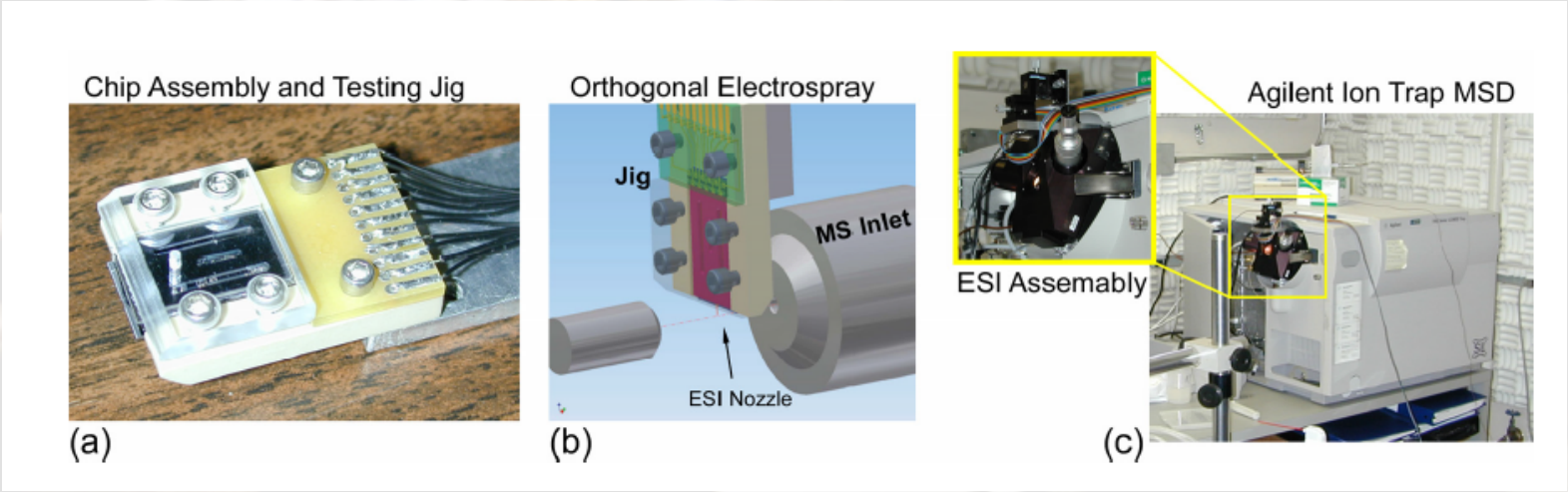

A20130412.OPJ
Frame 10 MS
Integrated Counts
m/z
H_3O^+, m/z=19
O_2^+, m/z=32
$(CH_3)CO^-$, m/z=43
$C_2H_5O^-$, m/z = 45
$C_2H_6OH^+$, m/z = 47
$(CH_3)_2CO^-$, m/z=58
$(CH_3)_2COH^+$, m/z=59

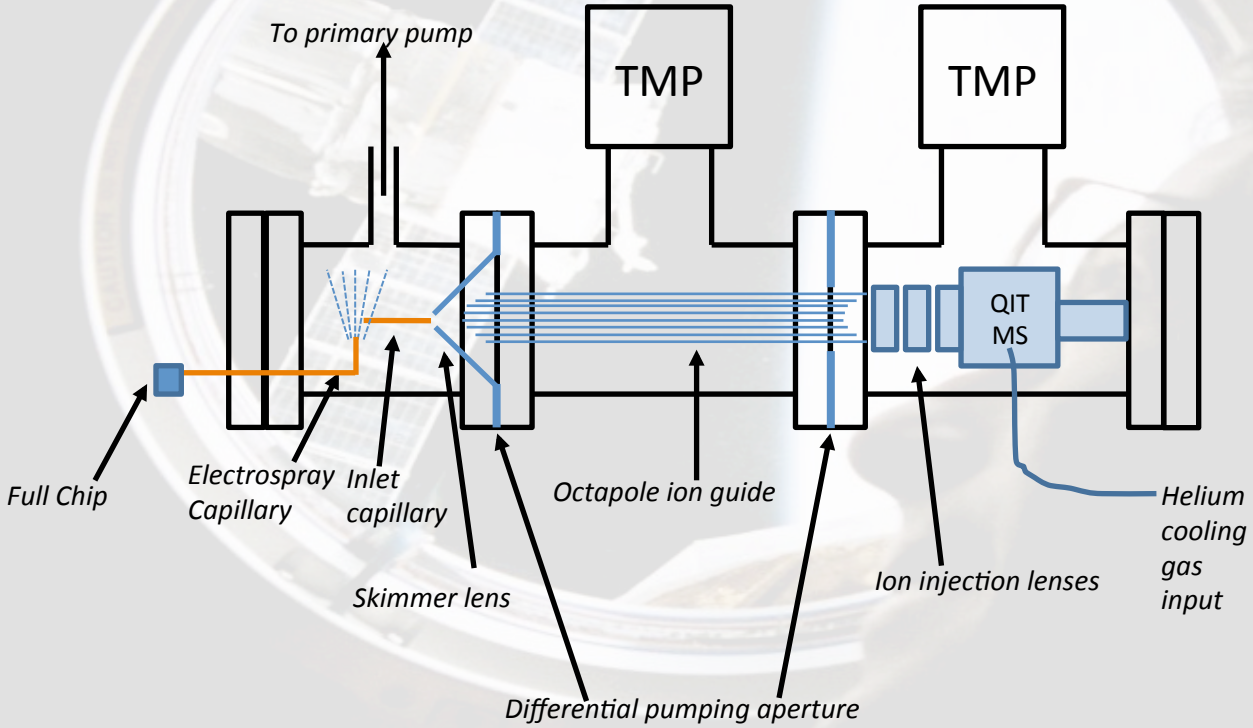

Total Ion Chromatograph for Water, Acetonitrile
Water (m/z = 18)
Acetonitrile (m/z = 39-42)
Counts
Time (s)

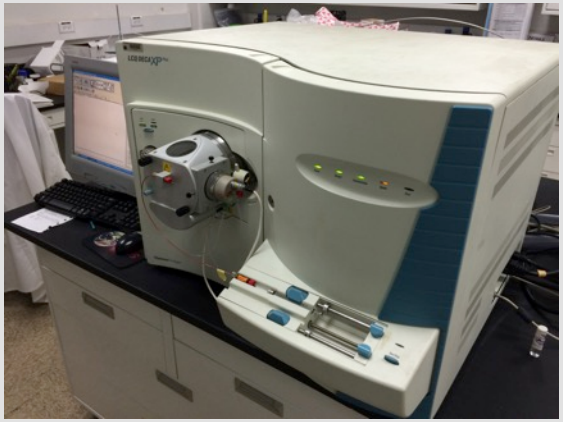

Integrated Counts
Mass43 Acetone
Mass45 Ethanol
Mass58 Acetone
Mass59 Acetone (R-H+)
Frame (1 sec per frame)

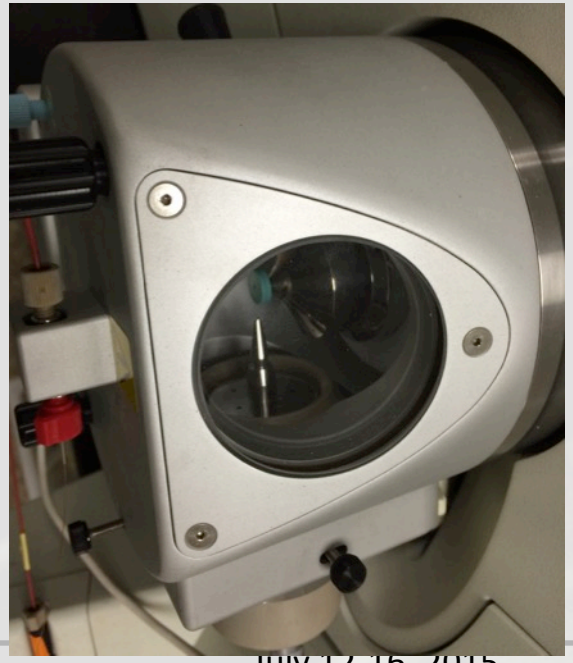

Centroided Counts for 250s Recording
Total Counts
Mass-Charge Ratio


To primary pump
TMP
TMP
Electrospray chip
QIT
MS
Octapole ion guide
Helium cooling gas input
Skimmer lens
Ion injection lenses
Inlet capillary
Differential pumping aperture


Chip Assembly and Testing Jig
(a)
Orthogonal Electrospray
Jig
MS Inlet
ESI Nozzle
(b)
Agilent Ion Trap MSD
ESI Assemably
(c)

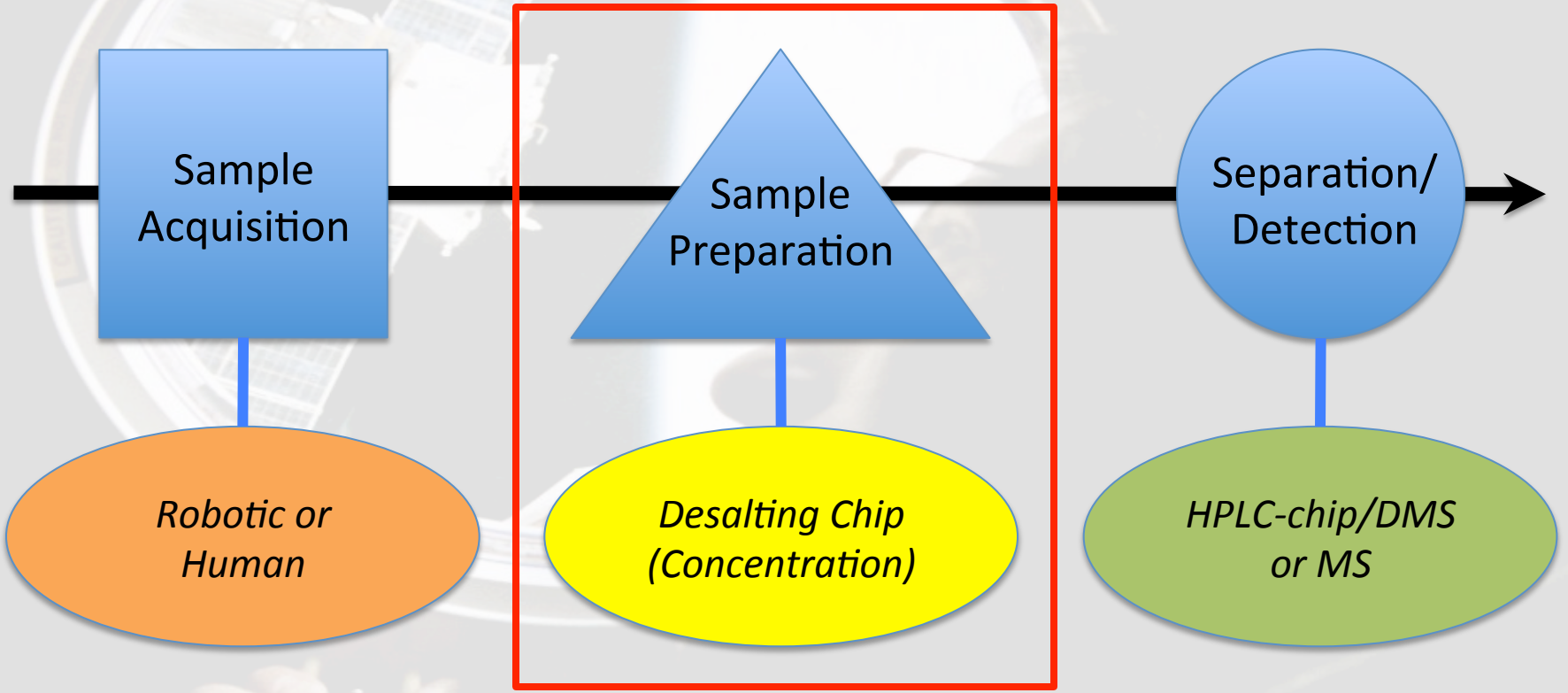






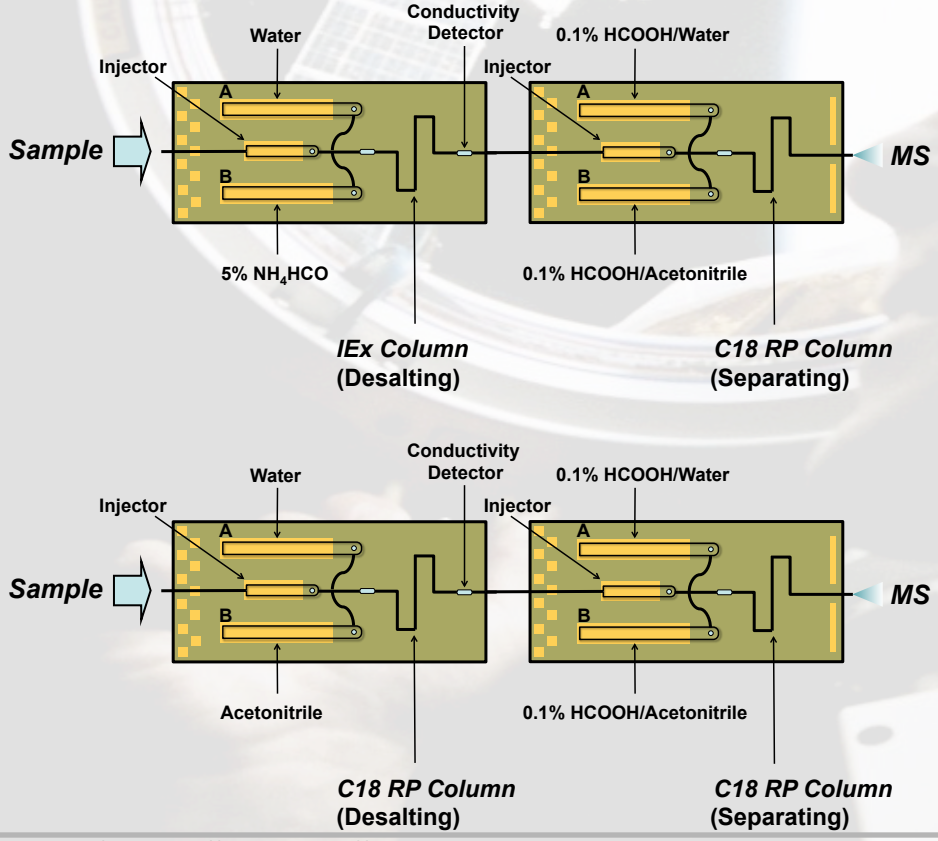
- **Capillary electrospray**
- **Traditional electrospray in vacuum (Thermo system), minimal pumping needed**
- **Minimizes vacuum sealing issues on chip side. Safest route**

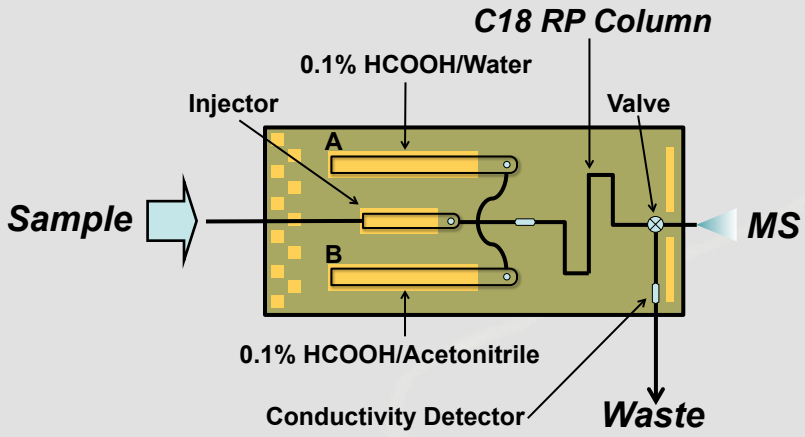
In designing *in situ*, liquid-based analytical instruments, three areas need to be addressed: *Sample Acquisition*, *Sample Preparation* and *Separation/Detection*.

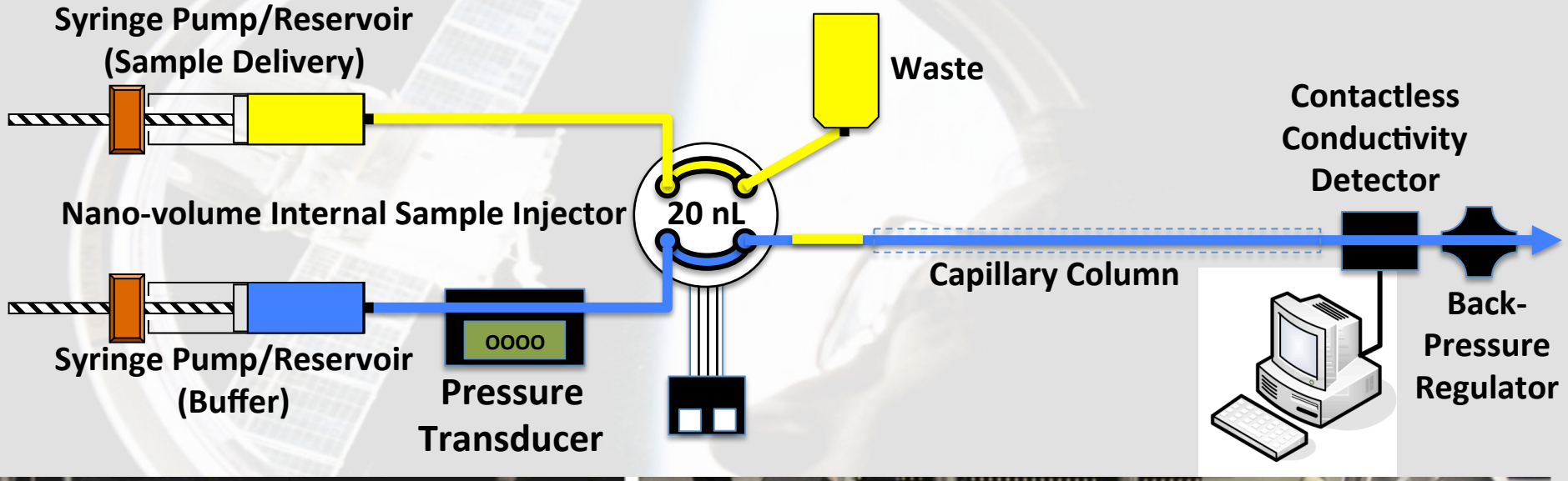


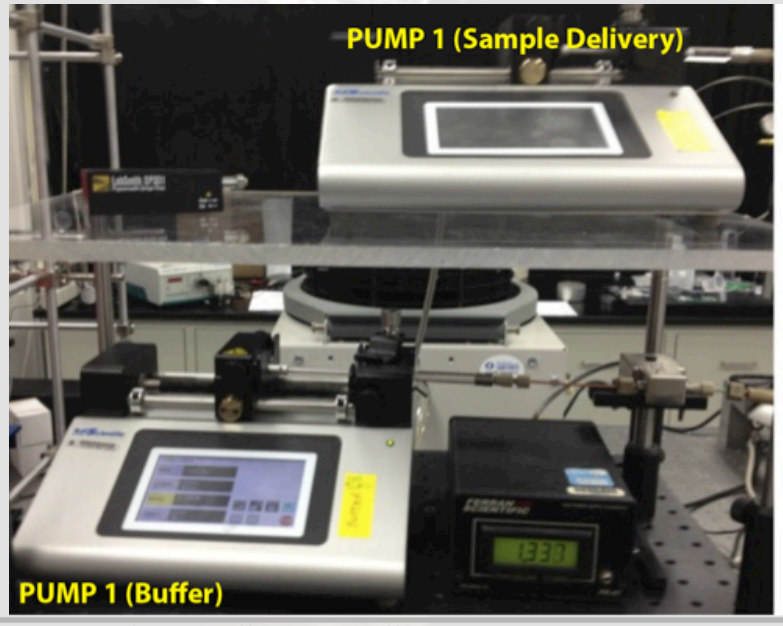
We are pursuing research into all three areas focusing, thus far, on developing a unique, miniaturized solute analyzer based on microfluidics technology. This analyzer consists of an integrated microfluidics High Performance Liquid Chromatographic-chips coupled to either a Differential Mobility Spectrometer (μHPLC-chip/DMS) or Paul Ion Trap Mass Spectrometer (μHPLC-chip/MS)

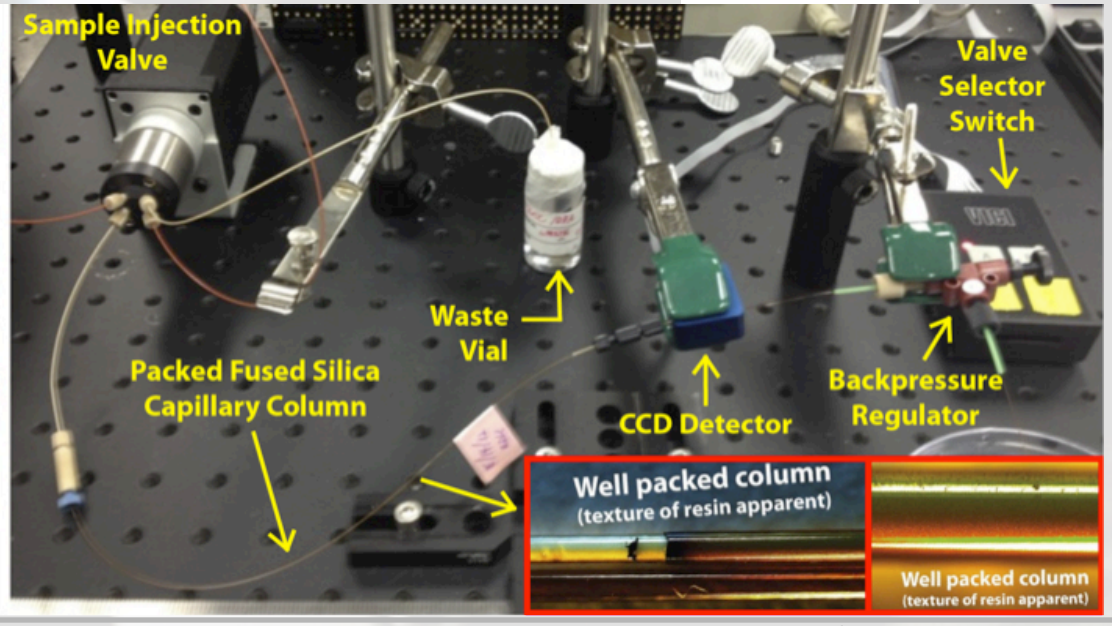
- One step that would be of critical relevance to the HPLC-chip/MS system is desalting; compound mixtures isolated from natural matrices often contain considerable amounts of nonvolatile salts.
- The presence of such salts may interfere with the operation of electrospray ion sources by clogging the skimmer and obscuring or suppressing ionization.
- in complex environments where high levels of salts are present, e.g. Mars (Boynton *et al.*, 2009; Hecht *et al.*, 2009), front-end devices may not be efficient enough (or dedicated enough) to desalt a sample for HPLC/MS.

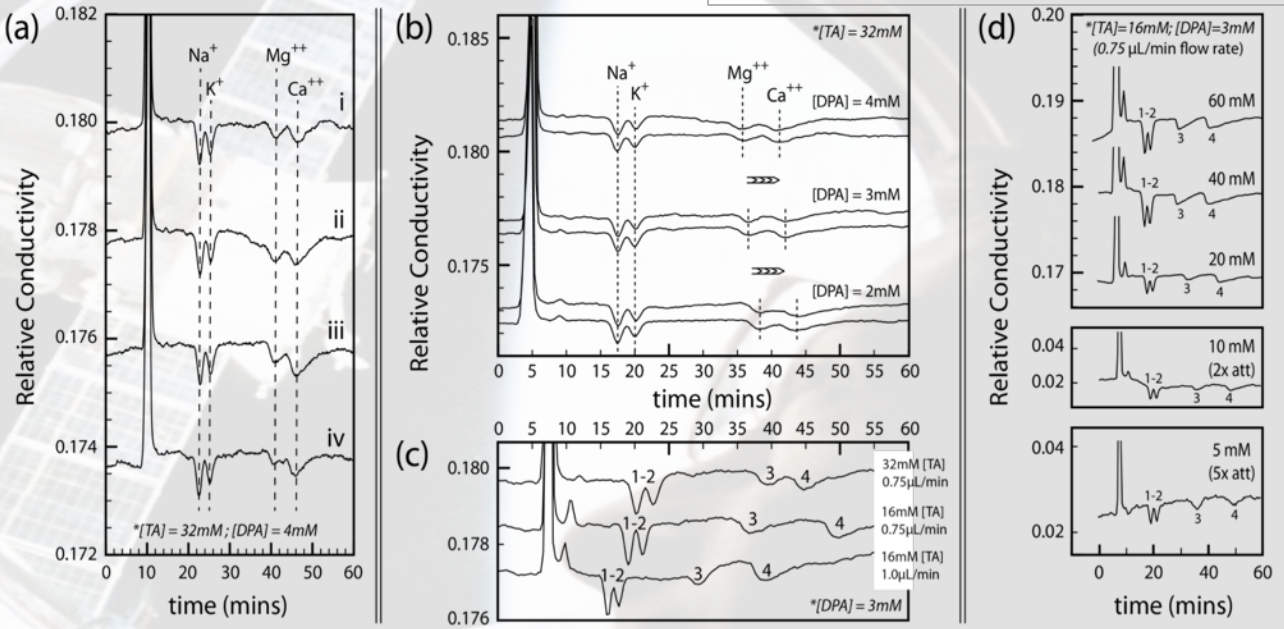





Syringe Pump/Reservoir
(Sample Delivery)
Waste
Contactless
Conductivity
Detector
Nano-volume Internal Sample Injector
20 nL
Capillary Column
Back-
Pressure
Regulator
Syringe Pump/Reservoir
(Buffer)
Pressure
Transducer


PUMP 1 (Sample Delivery)
PUMP 1 (Buffer)


Sample Injection
Valve
Valve
Selector
Switch
Waste
Vial
Packed Fused Silica
Capillary Column
CCD Detector
Backpressure
Regulator
Well packed column
(texture of resin apparent)
Well packed column
(texture of resin apparent)



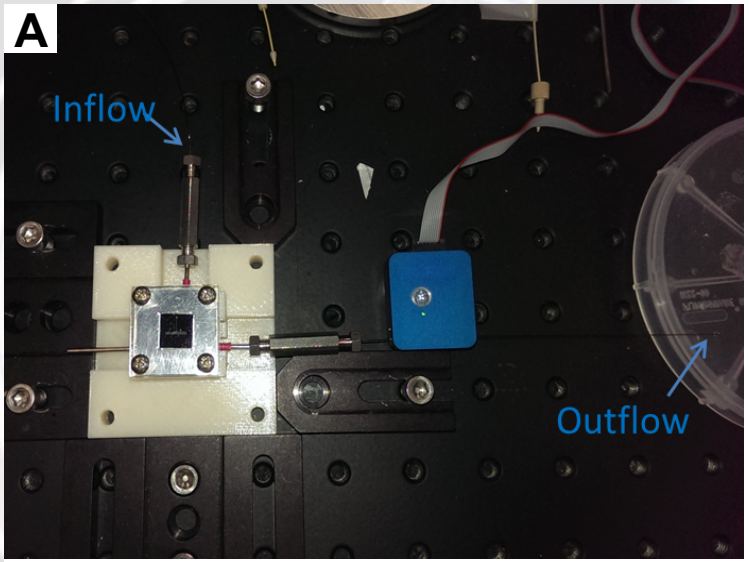
Test runs conducted to optimize separation conditions. *All experiments used tartaric acid (TA) as the eluting agent and dipicolinic acid (DPA) as a divalent complexing agent; 4-species mixtures were used for these experiments (Na^+, K^+, Ca^{2+}, Mg^{2+}). Capillary columns were packed with 30 µm diameter Phenomenex weak cation exchange resin, and pressures were between 0.7-1 MPa. Note that all runs include a system peak before any of the target cations appear – this feature is consistent with the literature.*

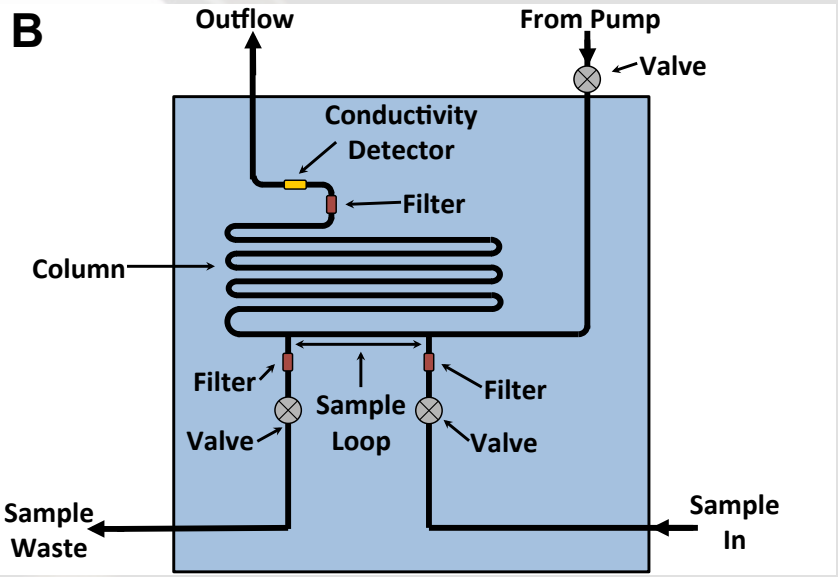
(a) Reproducibility tests (i – iv): four species resolved at 10 mM concentration (0.5 µL/min).

(b) Effect of [DPA] variability – increased concentrations of DPA reduce divalent retention times (0.5 µL/min).

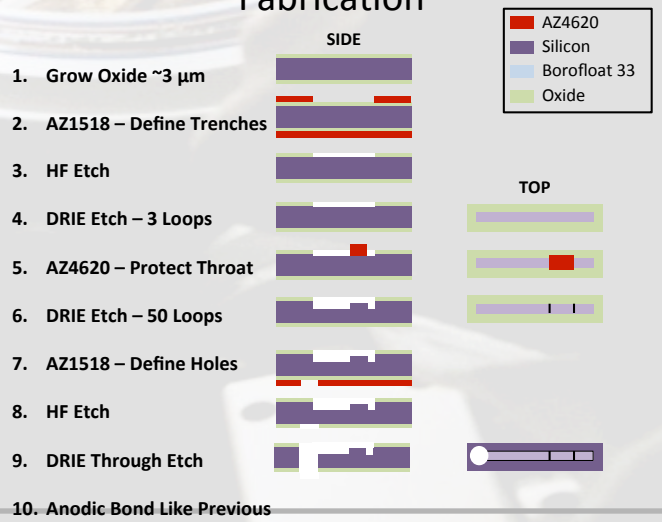
(c) (c)Effect of [TA] variability – shorter retention times are observed for divalent cations at higher [TA] at flow rates of 0.75 µL/min; a similar effect can be achieved using faster flow rates at lower [TA] (in this case 1.0 µL/min). While the run at 1.0 µL/min is shorter than the 0.75 µL/min run by ~10 minutes, the peak resolution benefits are minimal.

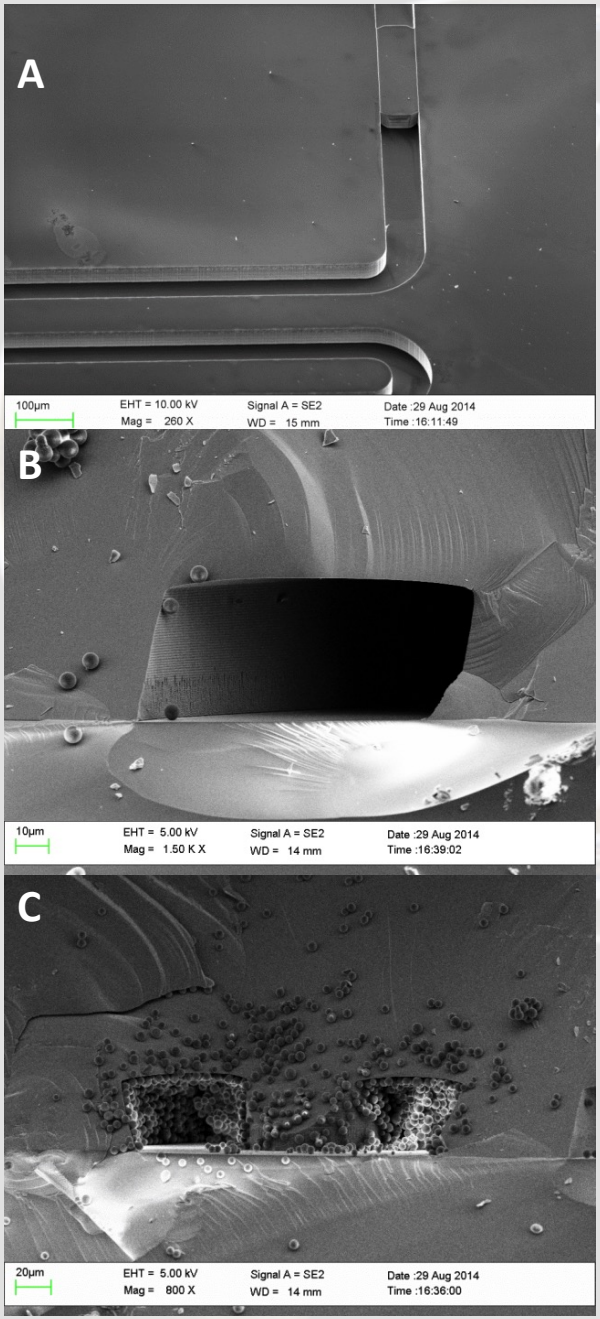
(d) Serial dilution from 5 mM to 60 mM demonstrating detection thresholds in the micromolar range based on the signal-to-noise ratio of the 5 mM run. The sensitivity of the system is not as high as expected.

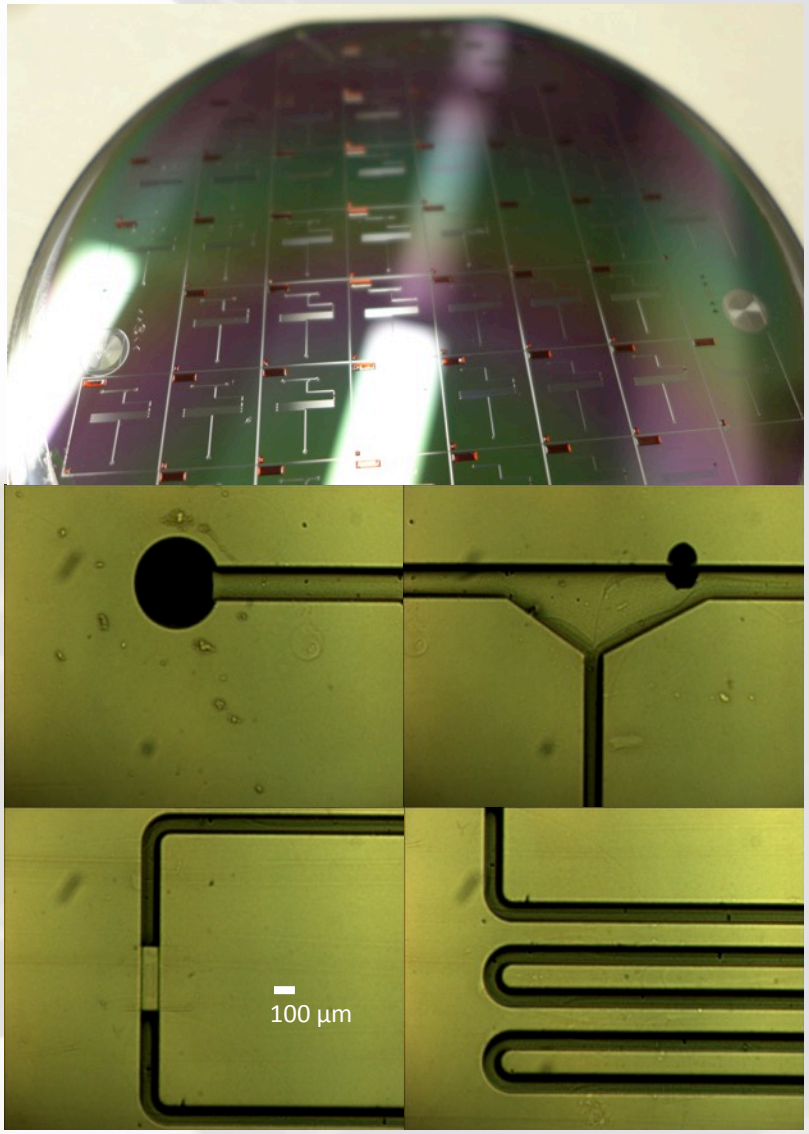




Fabrication




A
100µm
EHT = 10.00 kV
Mag = 260 X
Signal A = SE2
WD = 15 mm
Date :29 Aug 2014
Time :16:11:49
B
10µm
EHT = 5.00 kV
Mag = 1.50 K X
Signal A = SE2
WD = 14 mm
Date :29 Aug 2014
Time :16:39:02
C
20µm
EHT = 5.00 kV
Mag = 800 X
Signal A = SE2
WD = 14 mm
Date :29 Aug 2014
Time :16:36:00


100 µm

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